

administering an effective amount of a mutated LF protein or a fragment thereof and an effective amount of the *B. anthracis* protective antigen (PA) or an immunogenic fragment of the PA protein to the subject. In one embodiment, the immunogenic fragment of the *B. anthracis* protective antigen comprises consecutively amino acid 175 through amino acid 735 of the amino acid sequence shown in Figure 2B, i.e., amino acid 204 through amino acid 764 of SEQ ID NO. 4. A third method comprises administering a polynucleotide or nucleic acid comprising a sequence encoding *B. anthracis* LF protein or a fragment thereof to the subject. In one embodiment the polynucleotide which encodes the full-length mature LF protein comprises consecutively nucleotide 100 through nucleotide 2430 of the sequence, SEQ ID NO. 1, shown in Figure 1A. In one embodiment the polynucleotide which encodes an LF fragment comprises consecutively nucleotide 124 through nucleotide 855 of the sequence, SEQ ID NO:1, shown in Figure 1A. A fourth method comprises administering a polynucleotide which comprises a coding sequence for a mutated LF protein or immunogenic fragment thereof and a polynucleotide which comprises a coding sequence for the *B. anthracis* PA protein or an immunogenic fragment thereof to the subject. In one embodiment, the nucleotide sequence encoding the full-length, mature PA protein comprises consecutively nucleotide 88 through nucleotide 2295 of the sequence, SEQ. ID NO: 3, shown in Figure 2A. In one embodiment, the nucleotide sequence which encodes an immunogenic fragment of the PA protein, comprises consecutively nucleotide 610 through nucleotide 2295 of the sequence, SEQ ID NO: 3, shown in Figure 2A. The present methods stimulate or increase the level of antibodies which inactivate the *B. anthracis* lethal toxin in the subject.

✓ Page 4, paragraph beginning on line 17

Figure 1A shows a nucleotide sequence, SEQ ID NO:1, of a DNA which encodes wild-type *B. anthracis* LF protein; Figure 1B shows the amino acid sequence of the full-length mature, wild-type LF protein, i.e. amino acids 34 through amino acid 809 of SEQ ID NO. 2; Figure 1C shows the amino acid sequence of the polypeptide encoded by the eukaryotic expression plasmid pCLF4.

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Figure 2A shows a nucleotide sequence, SEQ ID NO.3, of a DNA which encodes a wild-type *B.*

anthracis PA; Figure 2B shows the amino acid sequence [SEQ ID NO.4.] of the full-length, mature wild-type PA protein [derived therefrom], i.e. amino acid 30 through amino acid 764 of SEQ ID NO. 4; and Figure 2C shows the amino acid sequence of the polypeptide encoded by the eukaryotic expression plasmid pCPA.

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In one embodiment the LF protein immunogenic fragment comprises amino acid 9 through amino acid 252 of the amino acid sequence shown if Figure 1B, i.e., amino acid 42 through amino acid 285 of SEQ ID NO. 2. The term LF protein fragment, as used herein, also encompasses LF protein fragments whose sequence differs from the sequence shown in Figure 1C. Such polypeptides have an amino acid sequence which is at least 90% identical, preferably at least 95% identical to the amino acid sequence, referred to hereinafter as the "LF protein fragment reference sequence", which begins with amino acid 9 and extends through amino acid 252 of the sequence shown in Figure 1B. Such variants, when injected into an animal, elicit production of antibodies that bind to the mature wild-type LF protein, i.e., the LF protein whose sequence is depicted in Figure 1B.

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In another aspect, the peptide-based immunogenic composition comprises a mutated LF protein or immunogenic fragment of LF protein and the *B. anthracis* PA protein or an immunogenic fragment thereof. The full-length, wild-type PA protein has a molecular weight of 83 kDa and comprises 735 amino acids. In one embodiment, the full-length, wild-type, mature PA protein comprises the amino acid sequence shown if Figure 2B, i.e. amino acid 30 through amino acid 764 of SEQ ID NO. 4. The term PA protein, as used herein also encompasses wild-type and mutated PA proteins whose sequence differs slightly from the sequence shown in Figure 2B. Such variants have an amino acid sequence which is at least 90% identical, preferably at least 95% identical to the amino acid sequence, referred to hereinafter as the "PA protein reference sequence" shown in Figure 2B. Suitable variants elicit production of antibodies that bind to the wild-type PA protein, i.e., the PA protein whose sequence is shown in Figure 2B.

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In one embodiment the PA protein fragment comprises amino acid 175 through amino acid 735 of the amino acid sequence shown in Figure 2B, i.e., amino acid 204 through amino acid 764 of SEQ ID NO. 4. The term PA protein fragment, as used herein, also encompasses proteins whose sequence differs slightly from the sequence shown in Figure 2C. Such variants have an amino acid sequence which is at least 90% identical, preferably at least 95% identical to the amino acid sequence, referred to hereinafter as the "PA protein fragment reference sequence", which begins with amino acid 175 and extends through amino acid 735 of the sequence shown in Figure 2B. Suitable variants of the PA fragment elicit production of antibodies that bind to the wild-type PA protein, i.e. the PA protein whose sequence is shown in Figure 2B.

Page 13, paragraph beginning on line2

The eucaryotic expression plasmid pCI (Promega, Inc.) was used to prepare a construct for the expression of a truncated version of the LF protein. The plasmid construct pCLF4 encodes the LF protein fragment consisting of amino acids 9-252 which includes the PA binding site. This plasmid was constructed from a PCR-amplified fragment using the primers 5'-CTGAAACCATCACGTAAAA-3' SEQ ID NO.5 and 3'-AGCAAGAAATAAATCTATAGTCTAGA-5' SEQ ID NO.6 which contain *Xba* cut sites. The *Xba*-digested PCR and pCI plasmid fragments were ligated to form the pCLF4 plasmid used in these studies. The resulting plasmid construct pCLF4 does not contain a signal sequence for secretion of the expressed gene product. All plasmids were purified from *E. coli* DH5 α using the Endo-free plasmid preparation kits (Qiagen) and resuspended in PBS before use.

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The eucaryotic expression plasmid pCI (Promega, Inc.) was used to prepare a construct for the expression of a truncated version of the PA protein. The gene fragment encoding amino acids 175-735 of the PA protein was PCR amplified using the plus strand primer (5'-CTCGAGACCATGGTT-3'; SEQ ID NO.7) and minus strand primer (3'-TAAGGTAATTCTAGA-5'; SEQ ID NO.8) using pYS2 as a template (Welkos 1988; Singh 1994). Included in the primer sequences are *Xho* and *Xba* restriction cut sites, respectively. The